Institut für Veterinärphysiologie
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Prof. h.c. Dr. med. vet. Max Gassmann

Arbeit unter wissenschaftlicher Betreuung von Dr. med. vet., PhD Melania Osto

Human-derived antibodies targeting IAPP aggregates for the treatment of Type 2 diabetes

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Kerstin Linder

Tierärztin
von Diepoldsau-Schmitter, Sankt Gallen

genehmigt auf Antrag von

Prof. Dr. med. vet. Thomas A. Lutz, Referent
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1 Zusammenfassung

Typ 2 Diabetes mellitus (T2DM) geht einher mit einer Reduktion der Anzahl Betazellen. Besonders toxische Oligomere, die bei der Bildung von IAPP-Ablagerungen entstehen, führen zu einer Schädigung von Betazellen.

Derzeit zielt keine der heutigen Behandlungsstrategien auf eine Verminderung der IAPP-Aggregation ab. Der humane Antikörper NI-203.26C11, der aggregiertes humanes IAPP bindet, bewies in vorhergehenden Studien mehrmals seine therapeutische Wirksamkeit in transgenen Ratten, die humanes IAPP exprimieren (RIPHAT). Dieses therapeutische Wirkprinzip wurde in dieser Studie mit einem zweiten Antikörper (NI-203.11B12) getestet. Zusätzlich wurde die therapeutische Wirksamkeit einer Kombinationstherapie mit NI-203.26C11 und Metformin evaluiert.

Mit NI-203.11B12 und NI-203.26C11 behandelte RIPHAT Ratten zeigten eine verbesserte Glucosetoleranz und eine erhöhte Insulinsekretion im Vergleich zu Kontrolltieren. Metformin verbesserte die Glukosetoleranz ebenfalls, jedoch ohne die Insulinsekretion zu verändern. Die Kombinationstherapie von Metformin und NI-203.26C11 führte auch zu einer verbesserten Insulinsekretion in RIPHAT Ratten. Somit scheint die Antikörpertherapie, wie auch eine kombinierte Behandlung, welche einerseits auf einer Verbesserung der Insulinsensitivität durch Metformin und andererseits auf dem Schutz von Betazellen durch die Antikörper NI-203.26C11 oder NI-203.11B12 beruht, sehr vielversprechend.

2 Summary

Human Type 2 diabetes mellitus (T2DM) is characterized by a reduction in functional beta-cells. Especially the toxic oligomers, which are produced during the aggregation of IAPP monomers into fibrils and amyloid deposits, damage the beta-cells.

At present, none of the current treatment strategies are aimed at the inhibition of the formation of IAPP aggregation. The human antibody NI-203.26C11, targeting aggregated human IAPP, showed its therapeutic efficacy in RIPHAT rats which express human IAPP, in previous studies. This therapeutic principle was evaluated in the current study with a second human antibody, NI-203.11B12. In addition, therapeutic efficacy of a combination therapy of the human antibody NI-203.26C11 and metformin was evaluated.

RIPHAT rats treated with NI-203.11B12 and NI-203.26C11 showed improved glucose tolerance and increased insulin secretion compared to controls. Glucose tolerance was also improved by metformin alone, but the combination with NI-203.26C11 also improved insulin secretion.

Thus, antibody therapy against toxic IAPP oligomers as well as a combined treatment based on an improvement of insulin sensitivity by metformin and the protection of beta-cells by the antibody NI-203.26C11 or NI-203.11B12 seems very promising.

3 Introduction

3.1 Overview

Human Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder and it is characterized by insulin resistance and impaired beta-cell function and mass. The origin of the disease is not yet fully understood, but it is thought to be the result of the interplay of genetic and epigenetic factors, and lifestyle factors such as obesity and reduced physical activity. Metabolic key features are hyperglycemia, an impaired lipid profile, and increased fatty acid oxidation. [1 2 3]

Similar to T1DM, important long-term consequences of the high glucose levels are micro- and macrovascular damage such as atherosclerosis, retinopathy, nephropathy and neuropathy. The severe complications in T2DM and the highly increased number of T2DM patients, which is probably linked to increased obesity rates, turned the investigation of new treatment strategies into a most relevant research field. [4 5]

3.2 Insulin resistance and Beta-cell dysfunction

Physiologically, beta-cells secrete insulin in a biphasic pattern after taking up a normal meal. [6] Insulin resistance, which occurs very early in T2DM development due to various factors such as obesity, less physical activity, and high calorie intake, all of which lead to an impaired glucose uptake in peripheral tissue such as muscle, liver and fat tissue, increases the demand for insulin. Hence, beta-cells try to compensate by releasing more insulin which often leads to overt hyperinsulinemia. In the beginning, this compensatory mechanism is still effective for most T2DM diabetics, but at a later stage the beta-cell capacity gets exhausted in people with a higher risk, the latter being influenced by genetic or environmental factors. [2 7 8]

When present, hyperglycemia itself can induce pro-apoptotic signals such as endoplasmic reticulum (ER) stress, mitochondrial dysfunction and thereby lead to beta-cell apoptosis which is part of the complex of glucotoxicity. Other features of T2DM are dyslipidemia with increased non esterified fatty acids, which may enhance oxidative stress in beta-cells (referred to as lipotoxicity), and changes in lipoproteins. These changes include increased low density-lipoproteins (LDL) and very low density-lipoproteins (VLDL) while high density-lipoprotein (HDL) which has been claimed to protect beta-cells, is decreased in T2DM. [9]

Besides gluco- and lipotoxicity, the formation of islet amyloid, derived of islet amyloid polypeptide (IAPP), also contributes to beta-cell loss. Since these IAPP aggregates occur to a much larger extent in T2DM patients, T2DM may also be considered a protein misfolding disease such as Alzheimer’s, Parkinson’s and Huntington’s diseases, obviously with a different peptide moiety that forms the pathological aggregates in pancreatic islets. [10]

3.3 Pancreatic islet amyloid formation

Pancreatic islet amyloid can be found in over 90% of T2DM patients, while it was only found rarely in older non-diabetic people. [11] Physiologically, IAPP, which is derived from an 89-amino acid (aa) precursor prepro-IAPP, is stored with insulin in the beta-cells. When glucose levels rise, insulin and IAPP are co-secreted from the beta-cells in a ratio of 100:1. Monomeric IAPP (also called amylin) serves as a satiation hormone and mediates an inhibition of gastric emptying, postprandial glucose release, a reduction of food intake, and an increase of energy expenditure.[12 13 14 15]

Initially, the pathophysiology of IAPP-derived aggregates was explained by the amyloid hypothesis, which suggests that cytotoxicity is caused by mature amyloid. Newer evidence indicated that the apoptosis of the beta-cells is probably better explained by the so-called toxic oligomer hypothesis. Toxic oligomers which are formed intracellularly during aggregation of IAPP molecules into oligomers and fibrils, lead to a decline of the beta-cell mass. It has been proposed that amyloid formation depends on a dynamic balance of assembly and disassembly between aggregates, which range from mono-, to small and larger oligomers, and insoluble amyloid fibrils. Eventually, amyloid deposits may serve as a nucleus for toxic oligomers. [3 16 17]

Findings from Gurlo et al. (2010) suggest that toxic oligomers are formed intracellularly through the secretory pathway and escape degradation through damage in the endoplasmatic reticulum and cell membranes.[18] Usually, the IAPP is stabilized by insulin and the pH in the secretory granules in the beta-cells. In T2DM, the increased demand of insulin also leads to an increased production of IAPP. This causes an overload of the control mechanism of the cell such as unfolded protein response (UPR) and autophagy dysregulation. Misfolded IAPP oligomers may then damage the cell membrane, the ER, and thereby induce beta-cell apoptosis. In addition, these aggregates may cause changes in the mitochondrial membrane potential, which leads to development of reactive oxygen species. Indirectly, IAPP oligomers can also contribute to inflammation in the pancreas and damage in beta-cells. [10 16]

Besides in humans, pancreatic IAPP deposits have also been observed in nonhuman primates and cats. These species are also prone to develop a form of T2DM similar to humans. IAPP is highly conserved among different species, except the region from amino acid 20-29. Interestingly, rodents, which do not develop T2DM, possess three proline residues within the region 20-29, which prevent the formation of amyloid. [19 20 21 22]

3.4 Rodent model

The progression of T2DM is slow and in people, it usually takes several years to develop the entire pathology with all the consequences of the disease. In contrast, rodent models, which recapitulate the specific beta-cell pathology, show faster progression of T2DM, and also develop long-term consequences within a shorter period of time.

Different rodent models for T2DM exist which possess at least some features of T2DM. Because rodent IAPP is not amyloidogenic, transgenic animals which express human IAPP (hIAPP) allow studying the pathophysiology and possible treatments against pathological amyloid deposits in pancreatic islets, hence which prevent or stimulate the clearance of IAPP. [23 24]

A heterozygous rat model, called HIP rat or RIPHAT rat, which expresses hIAPP driven by a rat insulin II promoter, was developed by Butler et al. (2004). This model spontaneously develops a form of T2DM between five and 10 months of age which is characterized by the occurrence of islet amyloid and increased beta-cell apoptosis. However, homozygous RIPHAT rats show an early onset of T2DM without presence of visible islet amyloid, probably due to rapid beta-cell destruction. [25]

3.5 Treatment strategies in T2DM

3.5.1 Immunotherapy

Based on the importance of IAPP aggregation in the pathogenesis of T2DM, a treatment which addresses this feature would be of primordial relevance. In Alzheimer's, another protein-misfolding disease, the formation of misfolded protein amyloid-Beta protein leads to damaging effects in the neurons. Currently, one of the most promising strategies to treat Alzheimer's disease is the development of a passive immunotherapy targeting the amyloid-Beta protein. In T2DM, different research groups showed that the idea of passive immunotherapy may also be of therapeutic value. [26] [27] [29] [30] For a more in-depth discussion, see paragraph "4.3 Anti-IAPP antibodies".

3.5.2 Non-insulin dependent therapies of T2DM

3.5.2.1 Introduction of glucose-lowering treatments (excluding insulin)

Lifestyle modifications, e.g. diet, weight control and physical action, are essential to support oral anti-diabetic therapies, but are often not effective alone. The effectiveness of these oral therapies is dependent on the remaining insulin secretory capacity of the beta-cells. A large number of different glucose-lowering classes are in clinical use, like metformin, sulphonylureas (SU), thiazolidinedione (TZDs), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose-linked cotransporter 2 (SGLT-2) inhibitors, glucagon-like peptide 1 (GLP-1) receptor agonists, meglitinides and alpha-glucosidase inhibitors. The guidelines of the American Diabetes Association (ADA) (2017) recommend metformin as an initial pharmacologic therapy for treating T2DM. This recommendation of the ADA is supported by a comparative effectiveness meta-analysis study which showed that metformin generally lowers HbA1C levels approximately 0.9-1.1% compared to SU, TZDs, alpha-glucosidase inhibitors, and DPP4 inhibitors. Dual- or triple therapies are recommended if glycemic control is no longer achieved. An additive glucose-lowering effect was seen when metformin was administered with SU, meglitinide, TZDs or alpha-glucosidase inhibitor. A comparative effectiveness study showed that in general improved glucose-lowering effects were seen in combined treatments. [30] [31]

3.5.2.2 Pharmacokinetic mechanism of metformin

Metformin uptake in intestinal cells is presumably primarily regulated by plasma membrane monoamine transporter (PMAT, encoded by gene SLC29A4), which is expressed on the luminal side of the enterocytes. Beside the transporter PMAT, OCT3 (gene SLC22A3) may also transport metformin. On the basolateral side of the intestinal cell, OCT1 (gene SLC22A1) may transfer metformin into the intestinal blood vessels.

In the body, the hepatic uptake is regulated by the transporter OCT1 (SLC22A1) and probably by OCT3 (SLC22A2), which are both located on the basolateral side of the hepatocytes. Beside these transporters, metformin is thought to be excreted by multidrug and toxin extrusion protein 1 (MATE1, gene SLC47A1).

Metformin is removed from the body by the kidneys. Circulating metformin is taken up in the renal epithelial cells by OCT2 transporter, which is located on the basolateral membrane of the renal tubules. Metformin is then excreted into the tubular lumen by MATE1 and MATE2, which are both located in the apical membrane of the proximal tubule cells.

Some metformin may then be reabsorbed. Reabsorption of metformin may be mediated by OCT1 transporters which are expressed on the apical and subapical domain side of both the proximal and the distal tubules. Additionally, the transporter PMA, which is located on the apical membrane of renal epithelial cells, may reabsorb metformin, too. [32]

The average half-life of metformin in blood plasma is approximately 4 -9 hours, but was increased up to 14 hours in other compartments such as erythrocytes and gastrointestinal tract. [30] [31]

3.5.2.3 Pharmacodynamic mechanism of metformin in relation of T2DM treatment

Metformin accumulates in mitochondria, leading to an inhibition of the complex 1 of the respiratory chain and thus to a suppression of the ATP production, respectively an increased cellular adenosinmonophosphat : adenosintriphosphat (AMP : ATP) ratio. Gluconeogenesis, which is a very energy-intensive process, is subsequently suppressed. Furthermore, the administration of metformin also influences other targets in the mitochondria such as the inhibition of the mitochondrial glycerophosphate dehydrogenase (mGPD) from the glycerophosphate shuttle which also reduces gluconeogenesis. However, this inhibition does not contribute to the reduction of glucose.

The increased AMP:ATP ratio leads to an inhibition of the fructose-1,6-phosphase (FBPase) which results in a reduced gluconeogenesis. Beside this effect, an increased AMP : ATP ratio by metformin can activate the phosphorylation of the AMP-activated protein kinase (AMPK), which is a serine/ tyrosine phosphatase, and influences various pathways in glucose and lipid metabolism. Interestingly, the enzyme AMPK can not only be activated by an increase in AMP : ATP ratio, but also in other ways by metformin. However, AMPK leads to the inhibition of the fat synthesis and the increase of the fat oxidation which is mediated by an inactivation (direct phosphorylation) of acetyl-CoA carboxylase (ACC). In addition, AMPK influences the lipid metabolism by inhibiting the expression of lipogenic genes such as fatty acid synthase, S14, SREBP-1C or different others. [35] [36] T2DM patients, who are treated with metformin, showed an improved lipid profile e.g. reduced levels of total cholesterol, LDL and triglycerides. [37] Another important action of AMPK is the increase in glucose uptake in the skeletal muscle by increasing the GLUT4 translocation activity. [38]

To summarize, metformin treatment leads to an activation of AMPK-dependent and independent pathways, and thereby mediates long-term insulin-sensitizing effects. Besides the liver, the intestine may also be a target organ of metformin. The uptake of metformin in the enterocytes causes an increase in anaerobic glucose metabolism and thereby a reduction of glucose uptake. However, it was observed that the exposure of metformin to the duodenum leads to suppression of glucose production, which is mediated by the gut-brain-liver axis and thereby activates AMPK and GLP-1. [38 39]

Another known effect of metformin is a loss of bodyweight, which can be explained by a reduced food intake but also due to other factors, such as an increased peripheral use, reduced rate of carbohydrate uptake in the intestine and an improvement in insulin action. [39 41 42] Different studies have shown that metformin can suppress inflammation by improving metabolic changes in T2DM such as hyperglycemia, insulin resistance and dyslipidemia. Interestingly, metformin also contributes directly to the reduction of inflammation by suppression of pro- and inflammatory cytokines or inhibition of NFκB. [42 43 45]

Furthermore, it was also discussed in different studies that metformin influences the microbiome. One of this studies had shown that metformin support the gut population of Akkermansia spp., which is related to a reduction of inflammation in adipose tissue and postprandial hyperglycemia. [45 46]

3.6 Preliminary data with anti-IAPP oligomer antibody treatment

3.6.1 *In vitro* studies

The therapeutic efficacy of a passive immunization with a human-derived antibody targeting pathologically misfolded hIAPP was tested *in vitro* and *in vivo*. Initially, human-derived IgG1 antibodies from healthy elderly subjects, which targeted different forms of hIAPP under *in vitro* conditions, were selected and generated. Subsequently, *in vitro* studies were conducted to validate the affinity and selectivity of the antibodies toward hIAPP aggregations. The antibody NI-203.26C11 was shown to possess a high selectivity for the pathological hIAPP aggregates and no binding to monomeric hIAPP in healthy subjects. In addition, the antibody NI-203.26C11 showed a dose-dependent neutralization of the hIAPP in beta-cells and stimulation of the uptake of hIAPP by human macrophages. These preliminary studies had been conducted in hIAPP expressing, transgenic mice and rats in collaboration with Neurimmune AG, Schlieren.

3.6.2 *In vivo* studies

3.6.2.1 Mouse study

In the first *in vivo* study in collaboration with Neurimmune AG, the therapeutic efficacy of the selected human-derived antibody was tested in the homozygous mice model expressing hIAPP (FVB/N-TG(Ins2IAPP)RHF/SOEL/J). The mice were treated with the antibody NI-203.26C11-r or PBS over three months. The therapeutic efficacy was assessed by body weight gain and glucose tolerance tests during the study, and the hIAPP-derived amyloid load in the pancreas at the end of the study. The treatment showed no improvement in glycemic control, glucose tolerance, and no deceleration of the progression in this animal model. However, the antibody was assessed to be well-tolerated in mice and did not cause toxic symptoms or immunoreactions. The lack of therapeutic efficacy might be explained by the use of an inappropriate animal model, indeed the progression of amyloid formation developed very rapidly so that the time window for therapeutic intervention may have been too short in this diabetic mice model. [48]

3.6.2.2 Rat study I

The therapeutic efficacy of a chimeric version of the antibody (NI-203.26C11-r), which possesses human variable domains and rat igG2B constant regions to reduce antigenicity in rats, was tested in male hemizygous RIPHAT rats.
The antibody NI-203.26C11-r or PBS were administered weekly in male RIPHAT and wild type Sprague Dawley rats from the age of 12 weeks over the duration of 18 weeks. The therapeutic efficacy was assessed by oral glucose tolerance tests (oGTTs), which were performed one week before, as well as 8 and 12 weeks after treatment start. In addition, the fasting glucose was measured 4 and 12 weeks after treatment start. At the end of the treatment, the rats were sacrificed and pancreatic beta-cell area and islet amyloid was evaluated by immunohistochemistry. The glucose tolerance in the treated groups was significantly improved compared to the control group. In addition, insulin levels tended to be higher and hIAPP levels were significantly increased in the treated RIPHAT-groups compared to the control RIPHAT-groups. However, there were no beneficial effects on body weight, fasting blood glucose, or on mean islet and insulin beta-cell area. [49]

3.6.2.3 Rat study II

A second rat study, with prolonged treatment duration of 28 weeks, was performed in RIPHAT and wild type rats with antibody treatment starting at the age of 12 weeks. The rats received the NI-203.26C11-r, and isotype control IgG or PBS intraperitoneal injections weekly. As before, oGTT were performed monthly. After 28 weeks of treatment, improved glucose tolerance, reduced fasting glucose and normalized body weight gain were observed in RIPHAT rats treated with NI-203.26C11-r compared to the PBS control group. After 20 weeks of treatment, a hyperglycemic clamp was conducted, which showed significantly improved insulin secretion in RIPHAT rats treated with NI-203.26C11-r compared to RIPHAT rats treated with IgG. [50]

3.6.2.4 Rat study III

A dose response study with three different doses of the antibody NI-203.26C11-r and a control PBS-group was performed in RIPHAT rats and wildtype Sprague Dawley rats. The rats received 1 mg/kg, 3 mg/kg or 10 mg/kg, respectively, of the NI-203.26C11-r antibody weekly from the age of 12 weeks for a treatment period of 41 weeks. For the assessment of the therapeutic efficacy of the different dosages, body weight (BW) was measured weekly and oGTTs were performed monthly. The BW gain of the antibody-treated groups was significantly increased compared to PBS-treated group starting at 37 weeks of treatment until the end of the study; in other words, the BW gain of the 1 mg/kg group showed a significant increase compared to the PBS-treated group.
The glucose tolerance was improved in all antibody treated RIPHAT rats, but especially in the 1 mg/kg and 10 mg/kg antibody NI-203.26C11 group. At the end of the treatment, fasting glucose was significantly decreased in the RIPHAT rats receiving the antibody at a dose of 1 mg/kg or 10 mg/kg, but not at 3 mg/kg.
The analysis of the area under the curve (AUC) of insulin during the oGTT indicated that in RIPHAT rats, which received the antibody, the insulin curve was improved compared to the RIPHAT rats without treatment. In addition, fasting insulin levels tended to be increased in the antibody-treated groups compared to the PBS-group. However, no significant differences were observed in glucose tolerance, fasting glucose, AUC of insulin or fasting insulin between the different doses of the antibody NI-203.26C11.

At the end of the study, the animals were sacrificed and pancreatic islet area and beta-cell mass were assessed. Increased pancreatic islet and beta-cell area was observed in the antibody-treated groups. The clearance of pancreatic hIAPP aggregates paralleled by increased islet macrophage infiltration was observed by the immunohistochemical analysis of the pancreas. Hence, the treatment with the antibody NI-203.26C11 showed its therapeutic efficacy at different doses, with generally the 1 and 10 mg/kg doses being more efficient than the 3 mg/kg dose. [51]

3.7 Current study

In the current study, an additional human-derived antibody, NI-203.11B12, was tested. The goal was to evaluate NI-203.11B12 as a backup antibody to confirm that targeting IAPP aggregates is of therapeutic value. For more information about the anti-IAPP antibodies, see paragraph “4.3 Anti-IAPP antibodies”.

The second aim of the study was the evaluation of the therapeutic efficacy of a combination therapy of the human-derived antibody NI-203.26C11 and metformin. The antibody NI203.26C11, which targets toxic IAPP aggregates, proved already its therapeutic efficacy as a monotherapy in previous rodent studies. On the other hand, metformin, a frequently used first-line glucose-lowering drug, is very helpful in the beginning of T2DM treatment. Disadvantageous is that metformin does not exert a direct effect on the protection of beta-cells. Matveyenko et al. (2009) described that in RIPHAT rats treated with metformin, no preservation of the beta-cell content was observed. [52]

This lead to us to the hypothesis that a combined treatment, which targets the increase of the insulin sensitivity by metformin, and the protection of beta-cells by the antibody NI-203.26C11, may be a promising therapeutic approach in T2DM treatment.

4 Material and Methods

4.1 Animals and housing conditions

The study was conducted with 78 hemizygous transgenic male RIPHAT rats (Crl:CD (SD)-Tg (Ins2-IAPP) 1Pfi) and 10 wild type male Sprague Dawley rats (WT; Cr:CD (SD)); (Charles River Laboratories, Wilmington, MA, USA). Rats were kept in a temperature controlled room (21 ±1°C) on a 12:12 hour light/dark cycle (light phase from 2am to 2pm) with *ad libitum* access to standard chow (Extrudate 3436, KLIBA NAFAG, Kaiseraugst, Switzerland) and water. Rats were housed in standard cages (Type 2000P, 612x435x216) in groups of two or three animals per cage. Before starting any treatment, rats (six weeks old) were handled three times per week and given an adaption period of eight weeks before being randomized into treatment groups. The experiments were approved by the Veterinary Office of the Canton Zurich, Switzerland (authorization number 143/2015).

4.2 Study design

4.2.1 Injection protocol

At 13 weeks of age, before starting any treatment, a first oGTT was performed and the collected data served as a baseline (see paragraph ”4.5 Oral glucose tolerance test (oGTT)” for details). After the oGTT, groups were matched for rats’ average fasting glucose, glucose tolerance and body weight (BW). The RIPHAT and wildtype (WT) rats received a combination of intraperitoneal (i.p.) injections and medicated water treatments, which started at 13 and 14 weeks of age, corresponding to week 0 and week 1 of the study, respectively. The i.p. treatment was repeated weekly while medicated water was replenished twice weekly. BW was measured to monitor the rats’ health and to determine the volume of the i.p. injection.

The animals were allocated into different treatment groups: n_1 corresponds to the initial number of animals, while n_2 corresponds to the number of animals after complete exclusion or inclusion, which had to be performed due to several reasons (see ‘4.2.2 Excluded animals’).

All WT (n_1= 10, n_2=11) rats received non-medicated water and were injected i.p. with phosphate buffered saline (WT-H2O+PBS; PBS, 1.5 ml/kg, Gibco, Auckland, NZ). RIPHAT rats (n_1=78, n_2=64) were randomly allocated into three i.p. treatment groups. The i.p. treatments were: PBS, 1.5 ml/kg (RIPHAT- PBS; Gibco, Auckland, NZ; n_1=31, n_2=28), a rat chimeric version of the human anti-hIAPP antibody NI-203.26C11-r (RIPHAT-26C11; 1.5 ml/kg (3 mg/kg) BW; n_1=31, n_2=22) and a rat chimeric version of a different human anti-hIAPP antibody, the NI-203.11B12-r (RIPHAT-11B12, 1.5 ml/kg (3 mg/kg) BW; n_1=16, n_2=13).

In combination with the i.p. treatment, n_2=14 (n_1=15) of the RIPHAT-PBS and n_2=12 (n_1=15) of the RIPHAT-26C11 rats received water with added metformin (RIPHAT-MET+PBS and RIPHAT-MET+26C11, respectively). Based on this allocation, there were n_2=37 (n_1=48) RIPHAT rats which received water (RIPHAT-H2O) and n_2=26 (n_1=30) RIPHAT rats which received a Metformin solution (RIPHAT-MET; see table 1 and 2 for details and doses).

After 28 weeks of the study, a washout was performed; the treatment of the RIPHAT-MET+PBS and RIPHAT-MET+26C11 groups was stopped. The metformin administration was replaced with plain drinking water and the i.p. injections were replaced or continued with PBS injections in the RIPHAT-MET+PBS and RIPHAT-MET+26C11 groups. The treatment of the RIPHAT-H2O group was conducted for 35 weeks until the end of the study. At the age of 48 weeks, all animals were sacrificed.

4.2.2 Excluded animals

Seven animals were partially excluded ($n_{partially\ excluded}$) because of health problems during the experiment. Because the health problems could not be directly related to the treatments received, their glucose and insulin values were included in the statistics until the time of their exclusion. In detail, one rat of the RIPHAT-H2O+PBS group had to be euthanized after 34 weeks of treatment because of skin reactions at the injection site. In the RIPHAT-H2O+26C11 group, one rat died due to an obstruction of the lower urinary tract after 21 weeks of treatment and another one was found dead by an unknown cause 34 weeks after the start of the treatment. In the RIPHAT-H2O+11B12 group, two animals had to be euthanized after 10 and 32 weeks respectively due to a tumor, likely a lipoma which is not uncommon in older rats. Another rat of the RIPHAT-H2O+11B12 group developed peritonitis due to an injury by i.p. injection after 11 weeks. In the RIPHAT-MET+26C11 group, one rat died because of a lymphoma after 16 weeks of treatment.

At the end of the treatment seven rats had to be completely excluded from the experiment ($n_{excluded}=n_1-n_{2-}-n_{partially\ excluded}$) and one was newly allocated. Five RIPHAT rats (4 RIPHAT-H2O+26C11, 1 RIPHAT-MET+26C11) showed very low antibody titers and three RIPHAT rats were not correctly genotyped by the animal supplier (1 RIPHAT-MET+PBS, 1 RIPHAT-MET+26C11, RIPHAT-H2O). The incorrectly genotyped rat of the RIPHAT-H2O+PBS group was newly allocated in the WT-H2O+PBS group ($n_1=10$, $n_2=11$).

Groups	i.p. injection (1.5 ml/kg)	Drinking water	n_1	n_2	$n_{partially\ excluded}$
WT-H2O+PBS	PBS	water	10	11	0
RIPHAT-H2O+PBS	PBS	water	16	14	1
RIPHAT-H2O+26C11	NI-203.26C11-r	water	16	10	2
RIPHAT-H2O+11B12	NI-203.11B12-r	water	16	13	3
RIPHAT-MET+PBS	PBS	metformin	15	14	0
RIPHAT-MET+26C11	NI-203.26C11-r	metformin	15	12	1

Table 1: The allocation in the different treatment groups were based on i.p. injection, plain water or water medicated with metformin. n_1 corresponds to the initial number of animal, n_2 corresponds to the number of animals at the end of the study, while $n_{partially\ excluded}$ corresponds to animals, which were excluded at some time point after the beginning of the study.

4.3 Anti-IAPP antibodies

Antibodies were generated as described by L. Hugentobler (2017). Briefly, B cells from elderly, healthy humans were screened for antibodies which bound selectively to aggregated IAPP without binding to non-aggregated IAPP. During the screening for antibodies targeting aggregated IAPP, two antibodies with different amino acid sequences were identified, cloned and recombinantly produced in Chinese Hamster Ovary (CHO) cells. Upon production, the antibodies were characterized for their selectivity to aggregated IAPP in human diabetic and non-diabetic tissue by ELISA, by bio-layer interferometry and by immunohistochemistry.

The antibodies NI-203.26C11-r and NI-203.11B12-r were shown to bind to an overlapping conformational epitope by an epitope mapping analysis. Rat chimeric versions of these two antibodies were generated for the *in vivo* studies in rats in order to avoid an anti-human immune response. The rat chimeric antibodies are composed of IgG2b of a rat backbone and a human variable region.

L. Hugentobler (2017) described the assessment of the affinity and selectivity of NI-203.26C11 by using fluorescent and bright field images (See figure 1). [47 48]

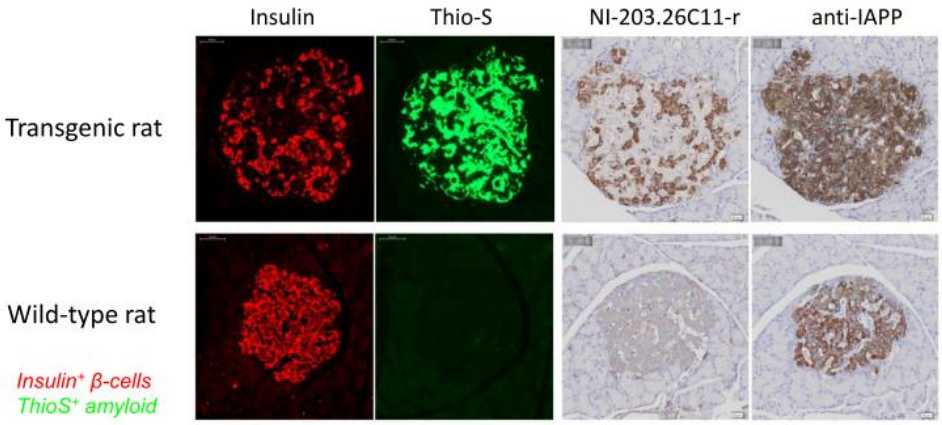


Figure 1: The assessment of the affinity and selectivity of NI-203.26C11: Fluorescent staining: The presence of amyloid (Thio-S)-positive pancreatic islets was confirmed in fluorescent images in RIPHAT but not in WT rats. Bright field images of the same pancreatic islets of RIPHAT and WT rats were stained with NI-203.26C11 and mouse anti-IAPP antibody. NI-203.26C11 staining was observed on amyloid (Thio-S)-positive islets from RIPHAT rats binding to aggregated hIAPP, but without binding to physiological rat IAPP (rIAPP) visualized by the mouse monoclonal anti-IAPP antibody on WT rat islets. Interestingly, binding of NI-203.26C11 antibody was also seen on Thio-S-negative areas in amyloid (Thio-S)-positive islets from RIPHAT but not WT rats (Description from L. Hugentobler (2017), Images from Neurimmune AG, Schlieren). [49 50]

4.4 Metformin

Metformin solution was replenished twice weekly by dissolving metformin (1,1-Dimethylbiguanide hydrochlorid Metformin, Sigma-Aldrich, Switzerland) in water. The administration of metformin was started after one week of the study (14 weeks of age) and lasted 28 weeks. The target dose of metformin was 200 mg/kg BW and the concentration of metformin (met conc; g/l) was, therefore, adjusted weekly between 3 to 3.8 g/l in drinking water. To let the animals adapt to metformin, the metformin concentration in the first two weeks of the study was 3 g/l and then increased to a maximum of 3.8 g/l (see table 2 and figure 2 for details).

The average water intake (avg WI, ml/kg) over two consecutive days was determined for the RIPHAT-H2O group, the RIPHAT-MET and WT-H2O group with the following formula:

$$\boldsymbol{avg\ WI} = \frac{\frac{\boldsymbol{wl}day0 - \boldsymbol{wl}day2 - \boldsymbol{l}}{\boldsymbol{w}} + \frac{\boldsymbol{wl}day1 - \boldsymbol{wl}day2 - \boldsymbol{l}}{\boldsymbol{w}}}{2}$$

The water content of the bottles (wl; ml) was measured over two consecutive days (day 0, day 1, day 2) and the difference described the water intake over 24 hours. For comparison, the difference of the water intake absorption was divided by the weight of the rats in a cage (w; kg). The water leakage (l; ml) describes the water loss by removing the bottles and was measured before the start of the study and was deducted during measurement.

To determine the average administered metformin dose (avg met dose; mg/kg BW) the following formula was used:

$$\boldsymbol{avg\ met\ dose} = \boldsymbol{met\ conc} * \boldsymbol{avg\ WI}$$

			average water intake (ml/kg BW/per cage/day)		
weeks of the study	**met conc (g/l)**	**avg met dose (mg/kg/day)**	**RIPHAT-MET**	**RIPHAT-H2O**	**WT-H2O**
1	3	181	60.29	80.21	76.36
2	3	173	57.77	77.48	78.57
3	3.5	236	67.29	82.67	84.07
4	3.5				
5	3.5	244	69.81	78.48	81.08
6	3.4	230	67.63	78.23	81.08
7	3.5	230	65.73	78.23	77.34
8	3.5	213	60.95	81.76	81.43
9	3.5				
10	3.5	201	57.52	68.86	67.44
11	3.5	223	63.72	77.33	67.98
12	3.5	194	55.34	66.93	65.48
13	3.5				
14	3.5	185	52.81	62.39	60.78
15	3.8	202	53.16	62.69	58.37
16	3.5				
17	3.5	207	59.24	62.64	57.45
18	3.5	204	58.23	65.30	63.89
19	3.5	205	58.61	67.81	67.45
20	3.5	180	51.48	68.24	67.45
21	3.5				
22	3.5	186	53.24	68.58	57.88
23	3.5	165	47.26	71.19	52.40
24	3.5	172	49.03	79.40	53.08
25	3.5	178	50.91	85.27	53.05
26	3.5				
27	3.5	161	46.09	89.60	49.65
28	3.5	173	49.46	96.45	48.85

Table 2: The average dose of metformin corresponding to the weeks of the study, metformin concentration (met conc), the average water intake (avg WI) of the treatment groups RIPHAT-MET, RIPHAT-H2O and WT-H2O groups.

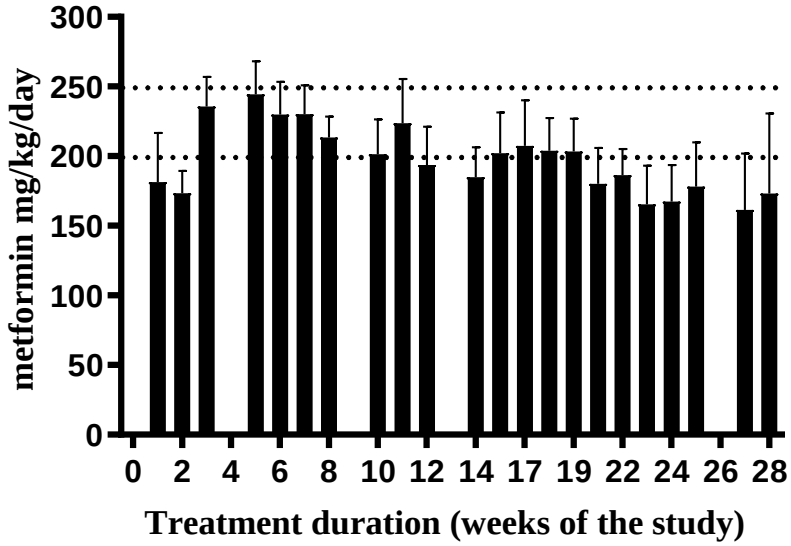


Figure 2: The administered average dose of metformin in the RIPHAT-MET groups over the course of the study. The lower dashed line corresponds to 200 mg/kg/day; the target dose. The upper dashed line corresponds to the upper dose limit of 250 mg/kg/day.

4.5 Oral glucose tolerance test (oGTT)

The first oGTT was performed before the beginning of the study, at 13 weeks of age. The following oGTTs were performed 4, 9, 13, 16, 21, 26, 30 and 34 weeks after the onset of the study.
Additionally, non-fasting glucose and insulin levels were measured at 18 weeks of treatment (5 hours into the light phase). Fasting glucose and insulin levels were measured after 12 hours fasting, 28 weeks after the beginning of the study.

Oral glucose tolerance tests were conducted as described in the dissertation of L. Hugentobler in 2017. [51] Following a 12 hour fast (from 7pm until 7am), BW was measured and rats received a 2 g/kg glucose solution (4 ml/kg BW of 50% glucose, B.Braun, Melsungen, Germany) by oral gavage. Rats were briefly anesthetized with isoflurane (3-4%, Attane, Piramal Enterprises Limited, Mumbai, India) and blood samples (250 µl full blood/500 µl EDTA Microtainer K2E tube, Becton Dickinson, Franklin Lakes, USA) were collected by sublingual sampling before (0 min) and 15, 30, 60 and 240 min after the glucose load. Blood glucose was measured using a Contour XT glucometer and glucose stripes (Contour, Bayer, Basel, Switzerland).

4.6 Plasma sampling and analysis

Plasma sampling was performed as described by L. Hugentobler (2017). The plasma insulin concentration was measured using a rat Insulin ELISA kit from Mercodia (Uppsala, Sweden) following the manufacturer's instructions.

4.7 Pancreas sampling

At the end of the study, the pancreas of the rats were collected as described by L. Hugentobler (2017). The rats were anesthetized with pentobarbital (60mg/kg, i.p.) and sacrificed by exsanguination via the V. cava caudalis at 48 weeks of age. At least 5 ml whole blood was collected and plasma was stored at -80°C. The pancreas was fixed in a 4% paraformaldehyde (PFA) solution for 24 hours at 4° C, then dehydrated in Shandon Citadell 2000 (Thermo Fischer Scientific, Waltham, USA) overnight. This was followed by embedding the pancreatic tissue in paraffin blocks (Leica EG1160, using paraplas from Leica Biosystems, Wetzlar, Germany), cut into 2.5 µm slices with a microtome (Leica RM2255, Leica Biosystems, Wetzlar, Germany), placed on glass slides and dehydrated overnight at 60°C.

4.8 Statistics

Graph Pad Prism 7 (San Diego, USA) was used for the statistics. For the analysis of fasting glucose, fasting insulin, area under curve (AUC) of glucose and insulin levels during oGTTs, a one-way analysis of variances (One-way-ANOVA) was used followed by Tukey's multiple comparison test. For the analysis of glucose and insulin values during the oGTTs and for bodyweight gain over time, a two-way analysis of variances (Two-way-ANOVA) was performed followed by Tukey's multiple comparisons test. For the analysis of the water consumption a one-way ANOVA was performed for each week followed by Bonferroni' test. A p-value < 0.05 indicated statistical significance. All data are presented as mean ± SEM.

5 Results

5.1 Animals

In the literature, the diabetic phenotype was observed to occur at the age 5 – 10 months. [25] Similarly, as observed in our previous studies, most of the RIPHAT-H2O+PBS rats of the current study developed polydipsia, polyuria and weight loss at the age of 9 months with few rats showing hyperglycemia. [51]

The RIPHAT rats treated with the antibody NI-203.26C11 and NI-203.11B12 and of the RIPHAT-MET groups showed less clinical signs of the diabetic phenotype compared to the control RIPHAT groups. However, after the washout that was performed at 28 weeks of study, the RIPHAT-MET groups started to develop diabetes-related symptoms similarly to the control RIPHAT groups. However, the RIPHAT-MET-PBS group showed a trend for a more severe progression and developed more severe clinical signs than the RIPHAT-MET+26C11 group.

number of oGTTs	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**wash out**	**8th**	**9th**	**Sac**
age (weeks)	13	17	22	26	29	34	39	41	43	47	48
weeks of the study	0	4	9	13	16	21	26	28	30	34	35
weeks of antibody treatment	1	5	10	14	17	22	27	29	31	35	-
weeks of metformin treatment	-	4	9	13	16	21	26	28	-	-	-

Figure 3: The timeline of the experiment with age in weeks, the corresponding weeks of the study, the corresponding weeks of antibody and metformin treatment from the 1st to the 9th oGTT and at sacrifice (sac). However, the antibody treatment started in week 0 of the study. The metformin treatment started 1 week after start of the study and was stopped after 28 weeks of the study, between the 7th and 8th oGTT (washout). For the washout, the metformin treatment was stopped and the i.p. injections with NI.203.26C11 in the RIPHAT-MET+26C11 group were stopped and continued with PBS injections. At this time-point an additional fasting glucose was measured.

Legend of used symbols

Legend of symbols used to indicate significant differences by one-way ANOVA excluding WT-H2O+PBS group

μ RIPHAT-H2O+26C11 vs. RIPHAT-H2O+PBS
λ RIPHAT-H2O+11B12 vs. RIPHAT-H2O+PBS
* RIPHAT-MET+PBS vs. RIPHAT-H2O+PBS
ω RIPHAT-MET+26C11 vs. RIPHAT-H2O+PBS
ρ RIPHAT-H2O+26C11 vs. RIPHAT-H2O+11B12
γ RIPHAT-H2O+26C11 vs. RIPHAT-MET+PBS
α RIPHAT-H2O+26C11 vs. RIPHAT-MET+26C11
κ RIPHAT-H2O+11B12 vs. RIPHAT-MET+PBS
τ RIPHAT-H2O+11B12 vs. RIPHAT-MET+26C11

The symbols also indicate the level of significance, e.g.:
RIPHAT-H2O+26C11 vs RIPHAT-H2O+PBS: μ: $p < 0.05$, μμ: $p < 0.01$, μμμ: $p < 0.001$; μμμμ: $p < 0.0001$

Legend of symbols used to indicate the different treatment groups in figures

WT-H2O+PBS
RIPHAT-H2O+PBS
RIPHAT-H2O+26C11
RIPHAT-H2O+11B12
RIPHAT-MET+PBS
RIPHAT-MET+26C11

WT-H2O+PBS
RIPHAT-H2O+PBS
RIPHAT-H2O+26C11
RIPHAT-H2O+11B12
RIPHAT-MET+PBS
RIPHAT-MET+26C11

Figure 4: The legend of symbols used for all graphs of the study. To simplify the graphs in the figures the significances were reported when $p < 0.05$. Only significances between RIPHAT groups were reported in figures and tables.

5.2 Body weight

The body weight was measured weekly in all rats during the entire study period. The RIPHAT and WT-H2O+PBS groups gained weight from the start until the end of the study. After 24 weeks of the study the BW gain of the different RIPHAT-H2O groups seemed to reach a plateau while the BW gain of the WT-H2O+PBS group increased until the end of the study. The BW gain of the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups did not differ significantly compared to the RIPHAT-H2O+PBS group or from each other.

The RIPHAT-MET groups showed a reduced BW gain compared to the RIPHAT-H2O groups from the start until 28 weeks of the study. These differences were significant at several time-points (see figure 5 and table 3).
The RIPHAT-MET groups were not significantly different between each other, but the BW gain of the RIPHAT-MET+26C11 group tended to be higher compared to the RIPHAT-MET+PBS groups from the begin until 28 weeks of the metformin treatment. After the washout in week 28 of the study, the increase of BW gain in the RIPHAT-MET+26C11 group was more pronounced compared to the RIPHAT-MET+PBS. The BW gain of the RIPHAT-MET+26C11 group increased and, after 29 weeks of the study, it was not significantly different compared to the RIPHAT-H2O. The BW gain of the RIPHAT-MET+PBS also increased but by far not to the same extent than the BW gain of the RIPHAT-MET+26C11 group.

To summarize, the rats of the RIPHAT and WT groups gained BW during the course of the study but with reduced BW gain in the RIPHAT-MET groups. The BW gain among the RIPHAT-H2O groups did not differ during the course of the study. The reduced BW gain under the metformin treatment is probably reflecting decreased food intake as observed in other studies. [42]

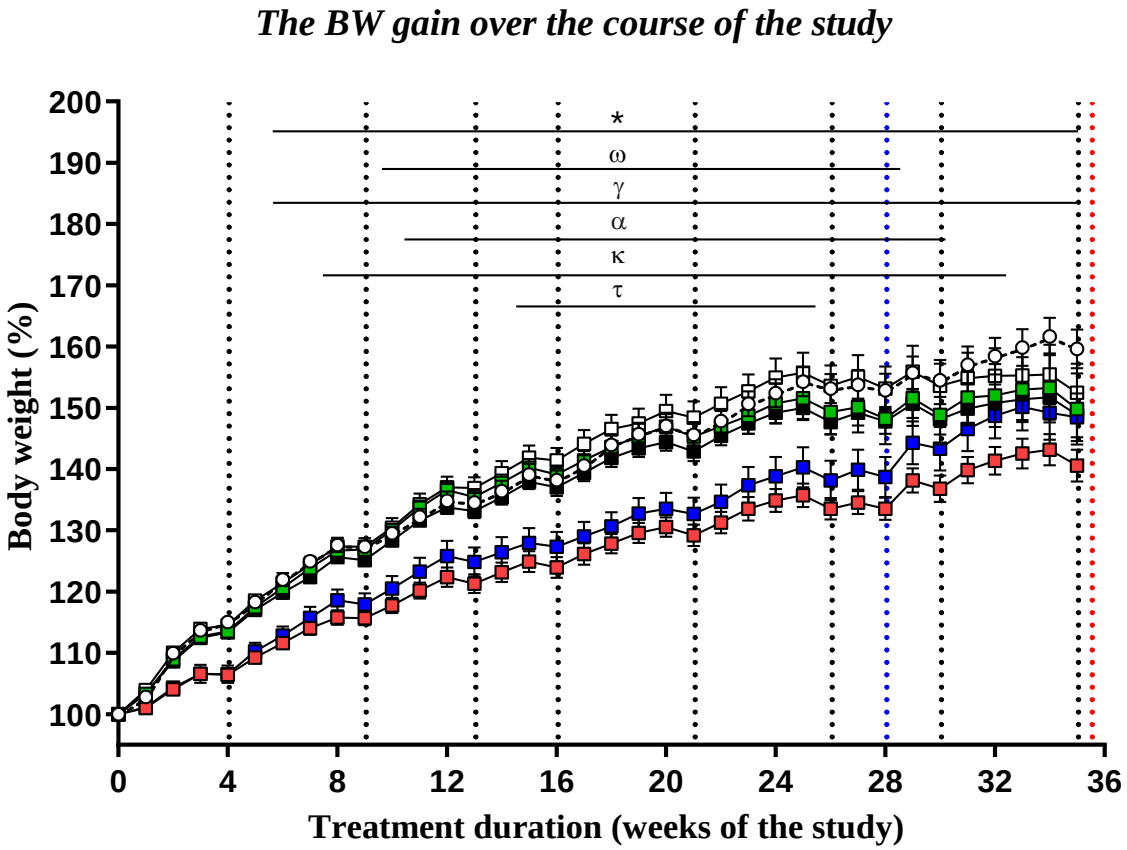


Figure 5: The body weight gain (BW gain; in % of starting body weight in week 0 set to 100%) of the WT-H2O+PBS and the different RIPHAT groups from onset of the study until sacrifice. The 1st to the 9th oGTT are marked with black grid lines, the washout is marked with a blue grid line, and the sacrifice is marked with a red grid line. See figure 4 and table 3 for more details. Only significances between the RIPHAT groups were reported.

weeks of study	WT-H2O+ PBS	RIPHAT-H2O+ PBS	RIPHAT-H2O+ 26C11	RIPHAT-H2O+ 11B12	RIPHAT-MET+ PBS	RIPHAT-MET+ PBS	Significances
0	100	100	100	100	100	100	ns
1	103	103	104	103	101	101	ns
2	110	109	110	109	104	104	ns
3	114	113	114	112	107	107	ns
4	115	114	115	113	106	107	ns
5	118	117	119	117	109	110	ns
6	122	121	121	120	112	113	* , γ
7	125	124	124	122	114	116	* , γ
8	128	127	127	126	116	119	** , γγ , κ
9	127	127	127	125	116	118	** , γγ , κ
10	130	130	130	128	118	121	** , ω , γγ , κ
11	132	134	134	132	120	123	*** , ω , γγγ , α , κκ
12	135	137	137	134	122	126	*** , ω , γγγ , α , κκ
13	135	135	137	133	121	125	*** , ω , γγγγ , αα , κκ
14	136	138	139	135	123	126	**** , ωω , γγγγ , αα , κκ
15	139	140	142	138	125	128	**** , ωω , γγγγ , αα , κκ , τ
16	138	139	141	137	124	127	**** , ωω , γγγγ , αα , κκκ , τ
17	141	141	144	139	126	129	**** , ωω , γγγγ , ααα , κκκ , τ
18	144	144	147	142	128	131	**** , ωω , γγγγ , ααα , κκκ , τ
19	146	145	148	143	130	133	**** , ωω , γγγγ , ααα , κκκ , τ
20	147	147	150	144	131	134	**** , ωωω , γγγγ , ααα , κκκ , τ
21	146	145	148	143	129	133	**** , ωω , γγγγ , ααα , κκκ , τ
22	148	147	151	145	131	135	**** , ωω , γγγγ , ααα , κκκ , τ
23	151	149	153	147	134	137	**** , ωω , γγγγ , ααα , κκκ , τ
24	152	151	155	149	135	139	**** , ωω , γγγγ , ααα , κκκ , τ
25	154	152	156	150	136	140	**** , ωω , γγγγ , ααα , κκκ , τ
26	153	149	154	148	134	138	**** , ωω , γγγγ , ααα , κκκ
27	154	150	155	149	135	140	**** , ω , γγγγ , ααα , κκκ
28	153	148	153	148	134	139	**** , ω , γγγγ , ααα , κκκ
29	156	152	156	151	138	144	**** , γγγγ , α , κκ
30	155	149	154	148	137	143	**** , γγγγ , α , κκ
31	157	152	155	150	140	147	*** , γγγ , κ
32	158	152	155	151	141	149	*** , γγγ , κ
33	160	153	155	151	143	150	** , γγ
34	162	153	156	152	143	149	** , γγ
35	160	150	152	149	141	148	** , γγ

Table 3: The body weight gain (BW gain; in % of starting body weight in week 0 set to 100%) of the WT-H2O+PBS and the RIPHAT groups over the course of the study. See figure 4 for the legend of significances between the RIPHAT groups. Only significances between RIPHAT groups were reported.

5.3 Fasting glucose

The fasting blood glucose (FG) levels were measured first in week 0 of the study. No significant differences were observed between the RIPHAT and WT groups in the absence of any treatment.

After the start of the treatment, the FG of the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups were significantly decreased compared to the RIPHAT-H2O+PBS group at several time-points during the study (see table 4). No significant differences were observed between the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 during the entire study.

FG was not significantly different among the RIPHAT-MET groups. However, RIPHAT-MET groups showed reduced FG compared to the RIPHAT-H2O+PBS at several time-points during the course of the study (see figure 6 and table 4).

In summary, the FG of the RIPHAT-H2O+PBS increased steadily over the course of the study. The FG of the RIPHAT-H2O+26C11, RIPHAT-H2O+11B12, RIPHAT-MET+PBS and RIPHAT-MET+26C11 groups were reduced compared to the RIPHAT-H2O-PBS. There was no significant difference observed between the antibody treatments in the RIPHAT rats.

weeks of study	WT-H2O+PBS	RIPHAT-H2O+PBS	RIPHAT-H2O+26C11	RIPHAT-H2O+11B12	RIPHAT-MET+PBS	RIPHAT-MET+26C11	Significances
0	8.15 ± 0.28	8.70 ± 0.23	8.10 ± 0.20	8.20 ± 0.32	7.59 ± 0.25	7.86 ± 0.25	ns
4	7.94 ± 0.32	8.48 ± 0.31	8.04 ± 0.21	7.91 ± 0.17	7.71 ± 0.20	7.95 ± 0.20	ns
9	7.58 ± 0.27	8.92 ± 0.30	7.80 ± 0.22	8.17 ± 0.20	8.01 ± 0.31	8.09 ± 0.19	μ
13	7.48 ± 0.30	8.25 ± 0.20	7.32 ± 0.16	7.24 ± 0.18	7.21 ± 0.19	7.59 ± 0.17	μμ, λλ, **
16	6.84 ± 0.19	8.05 ± 0.23	7.56 ± 0.26	7.46 ± 0.26	7.51 ± 0.21	6.95 ± 0.30	ω
21	7.02 ± 0.19	8.40 ± 0.24	7.72 ± 0.26	8.03 ± 0.19	7.80 ± 0.22	7.67 ± 0.21	ns
26	6.87 ± 0.14	8.49 ± 0.34	7.58 ± 0.20	7.64 ± 0.21	7.52 ± 0.19	7.77 ± 0.20	*
28	7.21 ± 0.18	9.58 ± 1.02	8.06 ± 0.65	7.39 ± 0.31	8.07 ± 0.43	7.68 ± 0.28	ns
30	7.25 ± 0.21	8.97 ± 0.61	7.62 ± 0.38	7.96 ± 0.24	7.81 ± 0.16	7.97 ± 0.23	ns
34	6.63 ± 0.13	10.71 ± 1.20	9.02 ± 0.92	8.98 ± 0.64	8.59 ± 0.61	8.28 ± 0.18	ns
35	10.95 ± 2.18	21.62 ± 2.56	21.16 ± 3.23	17.60 ± 1.84	18.53 ± 2.01	19.13 ± 1.83	ns

Table 4: The fasting glucose (FG) of the WT-H2O+PBS and the RIPHAT groups at different time-points over the course of the study. See figure 4 for the legend of significances between the RIPHAT groups. Only significances between RIPHAT groups were reported.

5.4 Fasting insulin

The fasting plasma insulin (FI) were measured first in week 0 of the study. The FI of the WT-H2O+PBS groups was significantly increased compared to the RIPHAT groups before the start of the treatment. No significant difference was observed among the RIPHAT groups before and after the beginning of the antibody treatment.

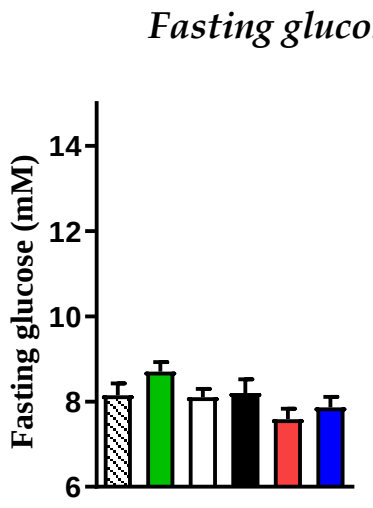
The FI of the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 tended to be higher compared to the FI of the RIPHAT-H2O+PBS over the course of the study. Interestingly, the FI of the RIPHAT-H2O+11B12 was significantly increased compared to the RIPHAT-MET+PBS after 9, 13, 16 and 28 weeks of the study.

The FI of the RIPHAT-MET groups did not differ significantly compared to the RIPHAT-H2O+PBS group. The FI of the RIPHAT-MET+26C11 tended to be increased compared to the FI of the RIPHAT-MET+PBS over the course of the study (see table 5 and figure 6).

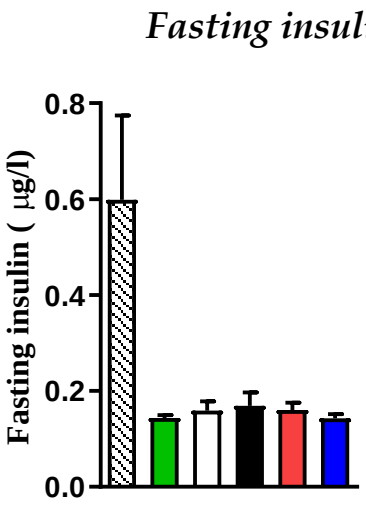
To summarize, the FI of the RIPHAT-H2O+26C11, RIPHAT-H2O+11B12 and RIPHAT-MET+26C11 was increased, while the RIPHAT-MET+PBS was comparable to RIPHAT-H2O+PBS.

weeks of study	WT-H2O+PBS	RIPHAT-H2O+PBS	RIPHAT-H2O+26C11	RIPHAT-H2O+11B12	RIPHAT-MET+PBS	RIPHAT-MET+26C11	Signifi-cances
0	0.67 ± 0.58	0.14 ± 0.02	0.16 ± 0.07	0.18 ± 0.12	0.16 ± 0.06	0.14 ± 0.03	ns
4	0.24 ± 0.09	0.17 ± 0.04	0.17 ± 0.03	0.18 ± 0.04	0.15 ± 0.02	0.17 ± 0.03	ns
9	0.18 ± 0.05	0.23 ± 0.12	0.21 ± 0.06	0.31 ± 0.15	0.18 ± 0.05	0.20 ± 0.07	ρ, κκ, τ
13	0.38 ± 0.39	0.17 ± 0.04	0.19 ± 0.04	0.24 ± 0.11	0.16 ± 0.03	0.21 ± 0.13	κ
16	0.21 ± 0.07	0.18 ± 0.03	0.21 ± 0.07	0.24 ± 0.10	0.16 ± 0.01	0.18 ± 0.04	λ, κκ, τ
21	0.30 ± 0.14	0.18 ± 0.04	0.20 ± 0.04	0.20 ± 0.04	0.17 ± 0.03	0.18 ± 0.06	ns
26	0.32 ± 0.19	0.20 ± 0.05	0.20 ± 0.04	0.21 ± 0.03	0.19 ± 0.03	0.21 ± 0.05	ns
28	0.47 ± 0.41	0.20 ± 0.04	0.24 ± 0.09	0.25 ± 0.05	0.18 ± 0.05	0.22 ± 0.07	κ
30	0.20 ± 0.07	0.17 ± 0.04	0.20 ± 0.06	0.20 ± 0.07	0.16 ± 0.04	0.20 ± 0.05	ns
34	0.20 ± 0.07	0.17 ± 0.05	0.20 ± 0.05	0.17 ± 0.04	0.16 ± 0.05	0.18 ± 0.05	ns
35	1.42 ± 1.06	0.18 ± 0.04	0.28 ± 0.13	0.21 ± 0.09	0.22 ± 0.11	0.21 ± 0.08	ns

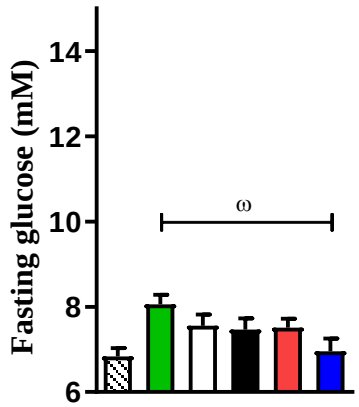
Table 5: The fasting insulin (FI) concentrations of the WT-H2O+PBS and different RIPHAT groups at different time-points of during the study. See figure 4 for the legend of significances between the RIPHAT groups. Only significances between RIPHAT groups were reported.



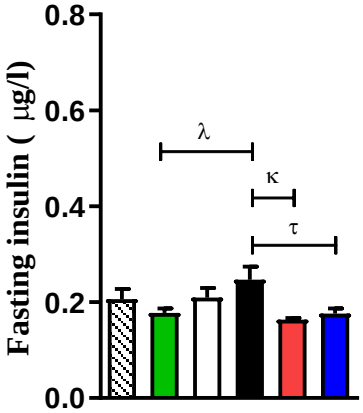
Before start of any treatment – 1st oGTT



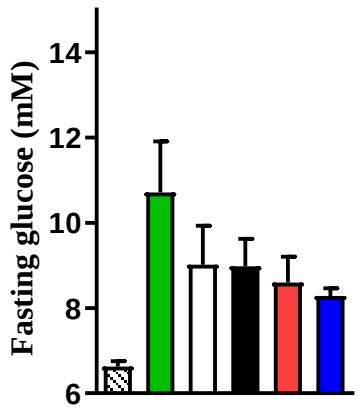
Before start of any treatment – 1st oGTT



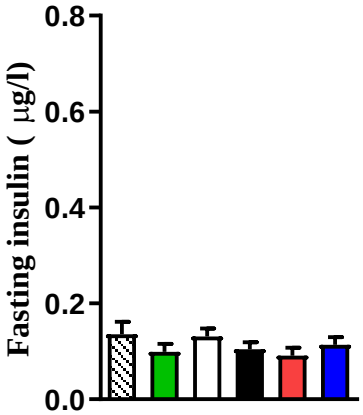
After 16 weeks of the study – 5th oGTT



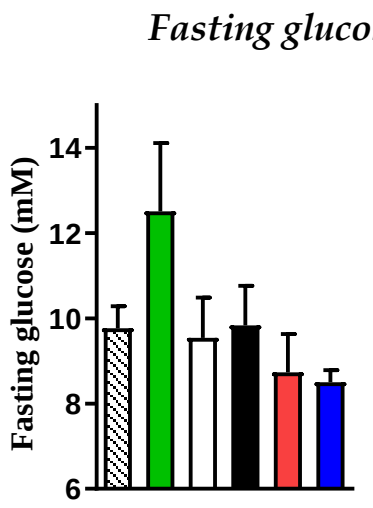
After 16 weeks of the study – 5th oGTT

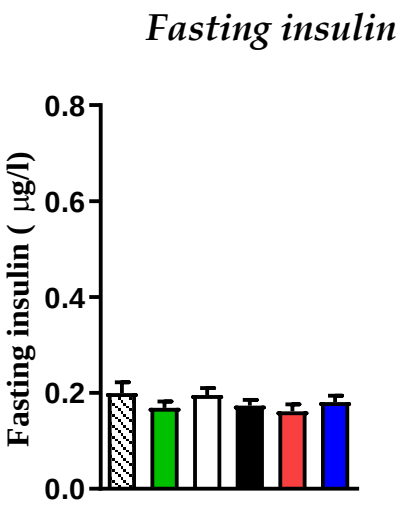


After 34 weeks of the study – 9th oGTT



After 34 weeks of the study – 9th oGTT





After 35 weeks of the study – 9th oGTT

After 35 weeks of the study – 9th oGTT

Figure 6: The fasting glucose and fasting insulin concentrations of the WT-H2O+PBS and the different RIPHAT groups before treatment in week 0 of the study (1st oGTT), after 16 weeks of the study (5th oGTT), after 34 weeks of the study (9th oGTT) and at sacrifice after 35 weeks of the study. See figure 4, table 4 and 5 for more details. Only significances between the RIPHAT groups were reported.

5.5 Glucose tolerance

Before the start of any treatment at the 1st oGTT and throughout the entire study, all RIPHAT groups showed an impaired glucose tolerance compared to the WT-H2O+PBS group. The baseline glycaemia and the AUC of glucose among the RIPHAT groups did not differ significantly before the beginning of the treatment.

However, the progression of glucose intolerance in the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups slowed down compared to the RIPHAT-H2O+PBS during the course study period. The glucose values and AUC of the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups were significantly decreased compared to the RIPHAT-H2O+PBS at several time-points. Based on glucose AUC and curves, the RIPHAT-H2O+26C11 and the RIPHAT-H2O+11B12 group did not differ significantly from each other.

The glucose tolerance and AUC of the RIPHAT-MET+PBS and RIPHAT-MET+26C11 improved compared to the RIPHAT-H2O+PBS during the entire study period. No significant differences were observed between the AUC of glucose and glucose curves of the RIPHAT-MET+PBS and the RIPHAT-MET+26C11 groups. Two weeks after the washout, the glucose tolerance of RIPHAT-MET groups was comparable to the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 group (see table 6 and 7, and figure 7 and 8).

To summarize, the glucose tolerance and AUC of the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups were improved compared to the RIPHAT-H2O+PBS. The glucose tolerance curve and the AUC of the RIPHAT-MET groups were improved compared to the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups. After cessation of the treatment in the RIPHAT-MET groups, the glucose tolerance of the RIPHAT-MET group was comparable to the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 group.

oGTT / weeks of study	time-point	WT-H2O+ PBS	RIPHAT-H2O+ PBS	RIPHAT-H2O+ 26C11	RIPHAT-H2O+ 11B12	RIPHAT-MET+	RIPHAT-MET+ 26C11	Significances
1. oGTT	0	8.1	8.7	8.1	8.2	7.6	7.9	ns
0 weeks	15	10.4	11.6	11.5	11.1	11.0	10.7	ns
	30	10.7	13.0	12.3	11.8	11.9	12.5	ns
	60	8.7	11.8	11.4	10.6	11.2	12.6	ns
	120	8.5	10.3	9.8	8.9	9.4	9.7	ns
	240	8.7	8.6	8.6	8.7	8.2	8.8	ns
2. oGTT	0	7.9	8.5	8.0	7.9	7.7	8.0	ns
4 weeks	15	10.3	12.2	11.1	10.4	10.9	10.6	ns
	30	10.0	14.0	12.3	11.0	11.2	11.2	λλλ, **, ωω
	60	8.4	13.4	11.7	10.3	10.9	10.8	λλλ, **, ωω
	120	8.9	11.0	10.0	9.3	9.0	9.1	*
	240	8.2	8.6	8.3	8.3	7.7	8.0	ns
3. oGTT	0	7.6	8.9	7.8	8.2	8.0	8.1	ns
9 weeks	15	10.7	12.8	12.3	11.7	11.5	11.8	ns
	30	10.5	14.9	13.6	12.8	12.0	12.2	*, ωω
	60	8.6	14.9	12.9	12.3	11.9	12.0	λ, **, ωω
	120	8.7	12.4	10.2	10.1	10.1	9.4	λ, ωω
	240	8.7	9.0	8.2	8.6	7.9	8.3	ns
4. oGTT	0	7.5	8.2	7.3	7.2	7.2	7.6	ns
13 weeks	15	10.9	14.6	13.4	12.3	12.5	11.9	ω
	30	10.2	17.2	15.2	13.8	13.3	13.4	λλ, **, ωωω
	60	8.7	18.1	15.3	13.8	13.8	13.5	λλλ, ***, ωωωω
	120	8.7	13.9	12.5	11.3	10.8	11.0	*, ω
	240	8.2	9.0	8.1	8.4	7.9	8.2	ns
5. oGTT	0	6.8	8.1	7.6	7.5	7.5	7.0	ns
16 weeks	15	10.2	15.5	13.6	13.1	12.3	11.8	**, ωω
	30	9.9	17.9	14.9	14.2	12.9	12.8	μ, λλ, ****, ωωωω
	60	8.1	18.8	16.0	14.9	13.4	12.4	μμ, λλλ, ***, ωωωω, α
	120	8.0	14.8	12.5	12.3	10.6	9.8	***, ωωωω
	240	8.0	9.1	8.1	8.6	8.3	7.8	ns
6. oGTT	0	7.0	8.4	7.7	8.0	7.8	7.7	ns
21 weeks	15	10.1	15.0	13.4	13.5	12.7	12.4	ns
	30	9.3	17.2	15.4	15.3	13.7	14.0	*, ω
	60	8.1	18.2	16.5	16.1	14.3	14.1	**, ωω
	120	7.8	15.4	13.9	13.2	10.8	10.7	***, ωωω
	240	7.9	11.1	8.6	8.8	8.6	7.7	ωω
7. oGTT	0	6.9	8.5	7.6	7.6	7.5	7.8	ns
26 weeks	15	9.8	16.1	13.5	13.2	12.7	12.4	**, ω
	30	9.2	17.9	15.1	15.5	13.4	14.4	*, ω
	60	8.3	18.5	15.8	16.2	14.2	15.0	*, ω
	120	7.5	16.2	13.5	14.0	12.0	11.7	*, ωω
	240	7.6	11.9	11.1	9.3	8.7	7.6	ωω

8. oGTT	0	7.2	9.0	7.6	8.0	7.8	8.0	ns
30 weeks	15	10.2	17.2	13.7	13.5	13.4	14.1	**
	30	9.5	19.6	15.0	16.3	14.0	16.3	ns
	60	8.2	20.4	17.2	17.4	15.3	17.1	*
	120	7.9	17.1	15.9	15.5	13.4	13.7	ns
	240	8.0	12.4	11.6	10.7	8.3	8.9	ns
9. oGTT	0	6.6	10.7	9.0	9.0	8.6	8.3	ns
34 weeks	15	10.0	19.1	16.2	15.5	15.3	15.0	ns
	30	9.2	21.4	18.7	18.2	16.8	17.5	ns
	60	8.2	22.1	19.9	19.4	18.0	19.4	ns
	120	7.5	18.4	17.7	16.1	15.6	15.9	ns
	240	7.6	13.4	14.1	11.9	11.8	11.3	ns

Table 6: The glucose levels of WT-H2O+PBS and RIPHAT groups during the oGTTs at 0, 15, 30, 60, 120 and 240 minutes. See figure 4 for the legend of significances between the RIPHAT groups. Only significances between RIPHAT groups were reported.

weeks of study	WT-H2O+PBS	RIPHAT-H2O+PBS	RIPHAT-H2O+26C11	RIPHAT-H2O+11B12	RIPHAT-MET+PBS	RIPHAT-MET+26C11	Signifi-cances
0	2139 ± 136	2502 ± 443	2422 ± 404	2295 ± 316	2328 ± 331	2468 ± 383	ns
4	2111 ± 131	2669 ± 478	2429 ± 377	2261 ± 372	2239 ± 371	2255 ± 306	ns
9	2144 ± 181	2921 ± 633	2536 ± 428	2506 ± 456	2415 ± 372	2397 ± 292	ns
13	2115 ± 141	3272 ± 619	2894 ± 682	2686 ± 548	2611 ± 543	2625 ± 560	ns
16	1987 ± 141	3421 ± 600	2922 ± 654	2865 ± 659	2586 ± 506	2430 ± 427	*, ω
21	1956 ± 136	3551 ± 976	3117 ± 800	3053 ± 779	2685 ± 578	2616 ± 373	*, ω
26	1902 ± 168	3716 ± 1185	3192 ± 1089	3157 ± 785	2786 ± 582	2757 ± 500	**, ωω
30	1986 ± 111	3962 ± 1297	3504 ± 1346	3455 ± 1015	2971 ± 572	3178 ± 729	**
34	1912 ± 157	4304 ± 1470	4064 ± 1614	3743 ± 1235	3594 ± 1057	3655 ± 785	ns

Table 7: The area under curve of glucose of WT-H2O+PBS and different RIPHAT groups at different time-points over the course of the study. See figure 4 for the legend of significances between the RIPHAT groups. Only significances between RIPHAT groups were reported.

5.6 Insulin levels

Plasma insulin values were measured during all oGTTs. The insulin response of the WT-H2O+PBS group was increased compared to the RIPHAT groups. No significant difference was observed among the RIPHAT groups before the start of the treatment, and after 4 weeks of treatment.

The insulin response of the RIPHAT-H2O+26C11 and RIPHAT-H2O+11B12 groups tended to be improved compared to the RIPHAT-H2O+PBS. The insulin values of RIPHAT-H2O+11B12 were significantly increased compared to the RIPHAT-H2O+PBS group at several time-points (see table 8).

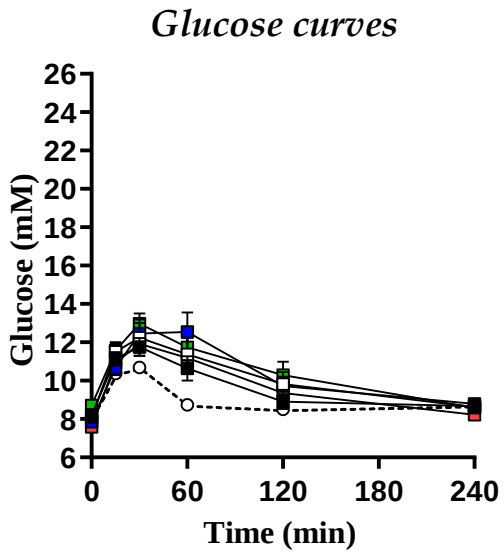
The insulin levels of the RIPHAT-MET+26C11 were improved compared to the RIPHAT-H2O+PBS group, and comparable to the insulin levels of the RIPHAT-H2O+26C11 and the RIPHAT-H2O+11B12 groups. After the washout was performed, the insulin levels of the RIPHAT-MET+26C11 seemed still increased compared to the RIPHAT-H2O+PBS group. The insulin response of the RIPHAT-MET+PBS group was not ameliorated before and after the washout, suggesting that metformin does not improve beta-cell function in RIPHAT rats.

To summarize, the treatment of the RIPHAT-H2O+26C11, RIPHAT-H2O+11B12 and RIPHAT-MET+26C11 groups improved the insulin response compared to the treatment of the RIPHAT-H2O+PBS group. However, the insulin levels of the RIPHAT-MET+PBS group showed no improvement and were comparable to the insulin levels of the RIPHAT-H2O+PBS group.

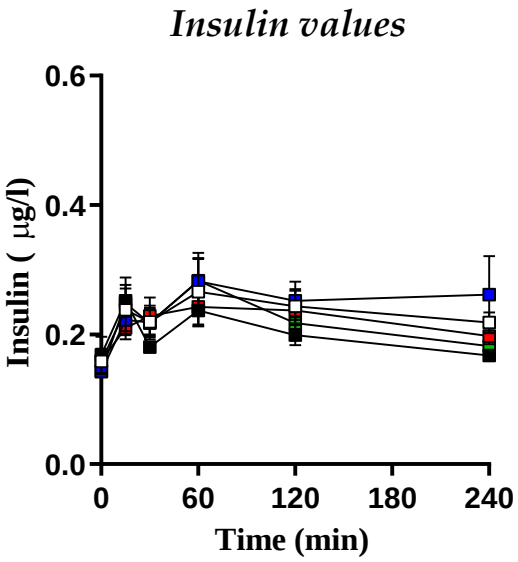
oGTT/ weeks of treatment	Time-point	WT-H2O+PBS	RIPHAT-H2O+PBS	RIPHAT-H2O+26C11	RIPHAT-H2O+11B12	RIPHA-MET+PBS	RIPHAT-MET+26C11	Significances
1. oGTT	0	0.60	0.14	0.16	0.17	0.16	0.14	ns
0 weeks	15	0.43	0.25	0.24	0.25	0.21	0.22	ns
	30	0.38	0.22	0.22	0.18	0.23	0.22	ns
	60	0.35	0.28	0.27	0.24	0.24	0.28	ns
	120	0.27	0.22	0.24	0.20	0.24	0.25	ns
	240	0.30	0.18	0.22	0.17	0.20	0.26	ns
2. oGTT	0	0.24	0.17	0.17	0.19	0.15	0.17	ns
4 weeks	15	0.49	0.29	0.35	0.34	0.27	0.33	ns
	30	0.40	0.24	0.28	0.32	0.21	0.25	ns
	60	0.37	0.27	0.34	0.32	0.25	0.30	ns
	120	0.31	0.26	0.30	0.33	0.24	0.28	ns
	240	0.33	0.15	0.16	0.18	0.22	0.16	ns
3. oGTT	0	0.18	0.23	0.21	0.33	0.18	0.22	ns
9 weeks	15	0.77	0.47	0.54	0.75	0.41	0.51	ρ, λλ, κκκ, ω
	30	0.36	0.25	0.32	0.38	0.21	0.24	ns
	60	0.44	0.35	0.44	0.48	0.23	0.37	K
	120	0.40	0.31	0.37	0.37	0.26	0.31	ns
	240	0.49	0.25	0.28	0.36	0.23	0.24	ns
4. oGTT	0	0.38	0.17	0.19	0.26	0.16	0.21	ns
13 weeks	15	0.73	0.27	0.28	0.38	0.23	0.29	ns
	30	0.62	0.24	0.28	0.39	0.18	0.22	κκ, τ
	60	0.51	0.30	0.39	0.48	0.21	0.25	λ, γγ, α, κκκ, ττ
	120	0.48	0.43	0.46	0.68	0.30	0.34	λλλ, ρρ, γ, κκκκ, ττττ
	240	0.53	0.28	0.28	0.36	0.18	0.30	κ
5. oGTT	0	0.21	0.18	0.21	0.25	0.16	0.18	ns
16 weeks	15	0.83	0.31	0.41	0.42	0.34	0.39	ns
	30	0.45	0.21	0.25	0.34	0.21	0.26	ns
	60	0.41	0.32	0.33	0.37	0.22	0.34	ns
	120	0.49	0.33	0.38	0.43	0.27	0.37	ns
	240	0.50	0.33	0.43	0.38	0.24	0.26	γ, α
6. oGTT	0	0.30	0.18	0.20	0.20	0.17	0.18	ns
21 weeks	15	1.09	0.31	0.38	0.32	0.27	0.34	γ
	30	0.69	0.26	0.32	0.29	0.24	0.31	ns
	60	0.53	0.26	0.43	0.33	0.27	0.36	μμ, γγ
	120	0.54	0.37	0.55	0.40	0.30	0.45	μ, γγγ
	240	0.61	0.32	0.46	0.36	0.27	0.31	μ, γγ, α
7. oGTT	0	0.32	0.20	0.20	0.22	0.19	0.21	ns
26 weeks	15	1.18	0.22	0.38	0.26	0.24	0.33	μ, γ
	30	0.68	0.23	0.34	0.32	0.21	0.29	ns
	60	0.50	0.25	0.34	0.30	0.24	0.33	ns
	120	0.63	0.32	0.44	0.43	0.27	0.44	γ, τ
	240	0.61	0.34	0.43	0.33	0.25	0.34	γ

8. oGTT	0	0.20	0.17	0.20	0.20	0.16	0.20	ns
30 weeks	15	0.69	0.21	0.35	0.28	0.24	0.27	ns
	30	0.51	0.21	0.30	0.30	0.20	0.26	ns
	60	0.58	0.21	0.40	0.30	0.22	0.30	μ, γ
	120	0.51	0.30	0.35	0.33	0.27	0.31	ns
	240	0.57	0.34	0.52	0.30	0.25	0.30	γγ
9. oGTT	0	0.20	0.17	0.20	0.17	0.16	0.18	ns
34 weeks	15	0.99	0.26	0.29	0.33	0.25	0.30	ns
	30	0.69	0.21	0.28	0.27	0.18	0.27	ns
	60	0.58	0.26	0.28	0.26	0.22	0.31	ns
	120	0.54	0.30	0.42	0.46	0.30	0.40	λ, κ
	240	0.53	0.37	0.42	0.40	0.33	0.35	ns

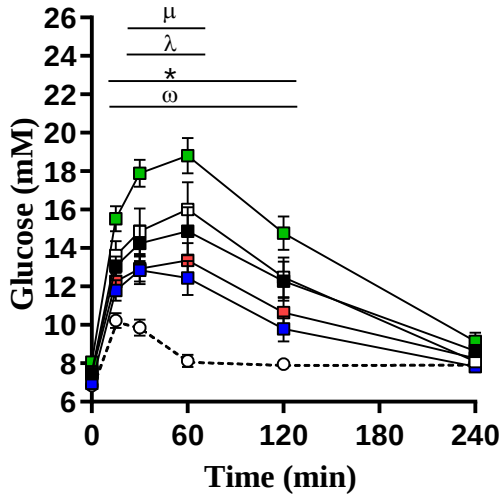
Table 8: The insulin levels during the oGTTs of the WT-H2O+PBS and different RIPHAT groups at 0, 15, 30, 60, 120 and 240 minutes. See figure 4 for the legend of significances between the RIPHAT groups. Only significances between RIPHAT groups were reported.



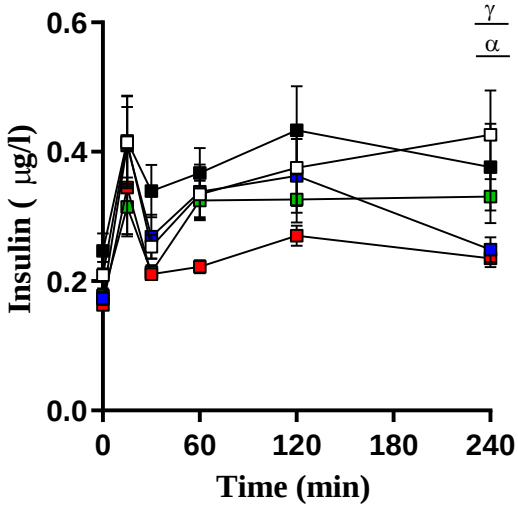
Before start of any treatment – 1st oGTT



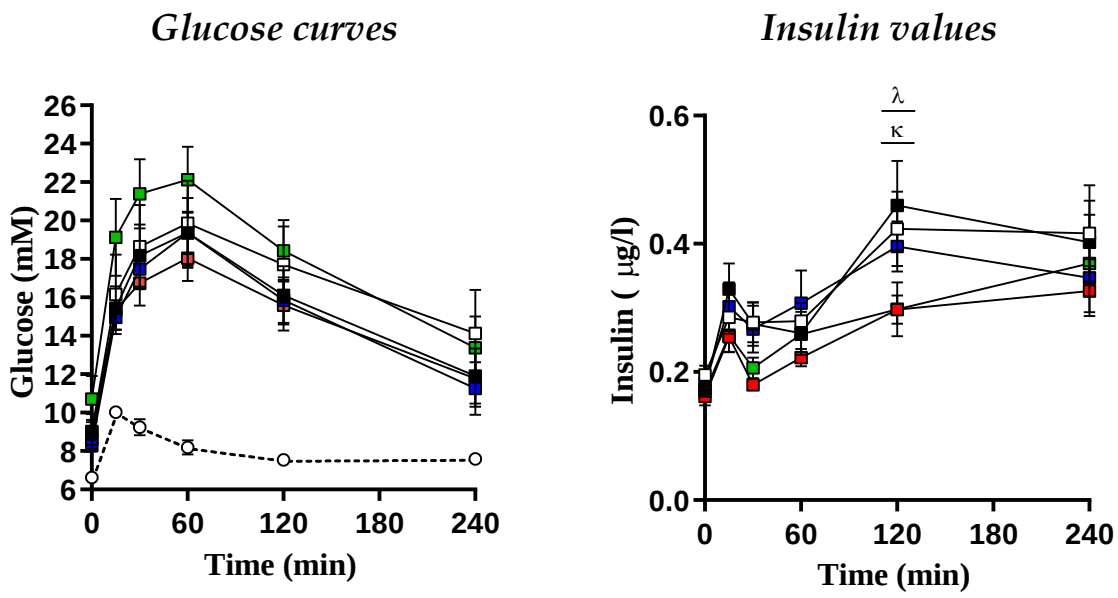
Before start of any treatment – 1st oGTT



After 16 weeks of the study – 5th oGTT



After 16 weeks of the study – 5th oGTT



After 34 weeks of the study – 9th oGTT

After 34 weeks of the study – 9th oGTT

Figure 7: The glucose curves and insulin levels of the WT-H2O+PBS and the different RIPHAT groups before treatment in week 0 of the study (1st oGTT), after 16 weeks of the study (5th oGTT), and after 34 weeks of the study (9th oGTT). See figure 4, table 6 and 8 for more details. Only significances between RIPHAT groups were reported.

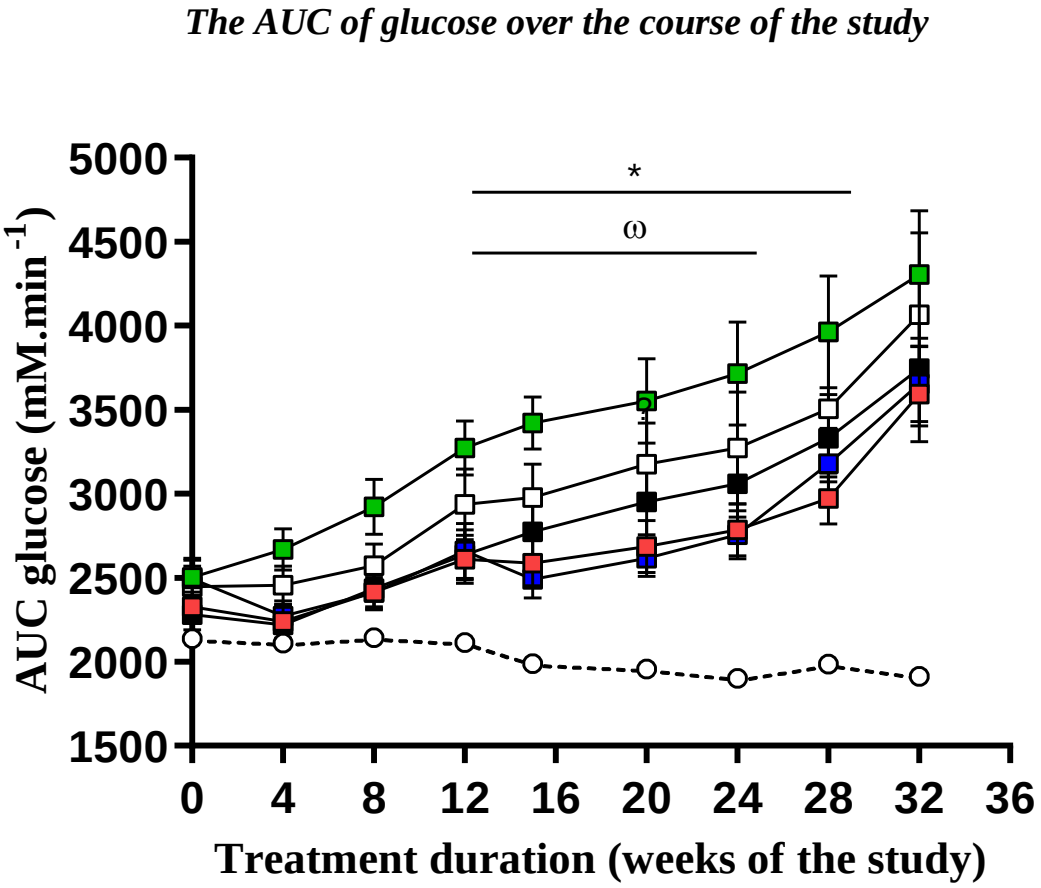


Figure 8: The AUC of glucose of the WT-H2O+PBS and the different RIPHAT groups at different time-points over the course of the study. See table 7 for more details. Only significances between RIPHAT groups are reported.

6 Discussion

The key characteristics of T2DM are insulin resistance and decreased beta-cell function, which result in an impaired insulin secretion. Various factors like glucotoxicity, lipotoxicity and IAPP-derived islet amyloid aggregates contribute to the development of T2DM. [1 3] The IAPP aggregation can be found in over 90 % of the patients of T2DM and is consequently discussed to play an important role in the development and progression of T2DM. In particular, the formation of toxic oligomers during intracellular IAPP aggregation is considered to lead to damage and loss of beta-cells. [3 11] Therefore, the prevention of the beta-cell loss and improvement of the beta cell function by targeting pancreatic IAPP aggregates is a valuable treatment strategy. In previous studies, the human-derived antibody NI-203.26C11, targeting toxic hIAPP oligomers, was assessed and proved to be a promising treatment against T2DM. [51]

In the current study, the therapeutic efficacy of a second human-derived antibody, the NI-203.11B12, targeting toxic hIAPP oligomers was evaluated in RIPHAT-rats. The antibodies NI-203.26C11 and NI-203.11B12 demonstrate both high affinity and selectivity towards pathological human IAPP aggregates in *in vitro* studies, despite differences in their amino acid sequence. These beneficial properties *in vitro* suggest that they have the same mechanism of action and hence the same treatment effect. Therefore, the therapeutic efficacy of the antibody Ni-203.11B12 was assessed and compared to a control group and the human-derived antibody NI-203.26C11.

A second goal of the current study was to evaluate the therapeutic efficacy of a combination therapy of the human-derived antibody NI-203.26C11 with metformin. Metformin is a first line therapy in the treatment of T2DM, which improves insulin sensitivity and hence lowers the glucose levels in diabetic patients. Although metformin has manifold beneficial effects, it was demonstrated that there is no effect of the preservation of the beta-cell content. [30 52] Therefore, we wanted to test the therapeutic efficacy of metformin combined with a human-derived antibody, which gives additional value by preventing the loss of beta-cells by toxic hIAPP oligomers.

For the evaluation of the different treatments, the RIPHAT-rat model was used. This model is characterized by an impaired glucose tolerance staring at three months of age and the development of a diabetic phenotype between five to ten months of age. The diabetic phenotype is mainly caused by defective insulin secretion and not by insulin resistance. In particular, the IAPP formation occurring in this model reflects an important aspect of T2DM pathogenesis in humans. Beside this, as the T2DM in humans, the progression of diabetes is slow in this model. Therefore, it is a suitable model to evaluate the therapeutic efficacy of the different treatments, especially of the human-derived antibodies NI-203.26C11 and NI-203.11B12. [25]

The antibody NI-203.26C11 had already been evaluated in several studies in RIPHAT rats in a collaboration with Neurimmune AG. The treatment with the antibody NI-203.26C11 was well tolerated and resulted in improved glucose tolerance, reduced hyperglycemia, enhanced plasma insulin values, preserved beta-cell content and normalized BW gain in RIPHAT rats. In the previous study, the antibody NI-203.26C11 was tested at the doses 1, 3 and 10 mg/kg over the course of 41 weeks. Irrespective of the dose, RIPHAT rats, which received the antibody NI-203.26C11, preserved their BW, while RIPHAT rats without treatment started to lose BW at the age of 37 weeks. At several time points, the fasting glucose was significantly

decreased compared to the RIPHAT-PBS group, while no differences between the different doses were observed. Based on glucose curves and AUC of glucose, the progression of glucose intolerance was reduced in all treatment groups. Fasting insulin and insulin values were improved by the antibody treatment, which may suggest an effect of the antibody in protecting beta-cell function. To summarize, the antibody NI-203.26C11 improved well-being, BW gain, glucose tolerance and insulin values at all three doses. [49 50]

In the current study, BW gain, fasting glucose, glucose levels, AUC of glucose, fasting insulin and the insulin levels were measured to evaluate the therapeutic efficacy of a treatment with the antibody NI-203.26C11 compared to the antibody NI-203.11B12, with metformin and with a combination treatment with the antibody NI-203.26C11 and metformin.

From the start until the end of the current study, all RIPHAT rats and WT rats showed increased BW gain. The BW gain of the RIPHAT-H2O+26C11 and the RIPHAT-H2O+11B12 increased and reached a plateau after 26 weeks of treatment. No difference was observed compared to the control group, which received only PBS injections and water for drinking. Hence, these findings about NI-203.26C11 were confirmed in the previous studies. In an even longer study period of 41 weeks of treatment, it was observed that the BW gain of the RIPHAT rats treated with NI-203.26C11 reached a plateau, too. However, in that study, the PBS control rats lost weight during the last 4 weeks of the study. [51] A difference in BW gain could also be expected if the current study would be prolonged. The RIPHAT rats, which received metformin, showed reduced BW gain compared to the other groups. This effect is most likely reflecting a reduced food intake which is known under metformin treatment. [37]
After 28 weeks of the study, the treatment of the metformin and the i.p. injections in the RIPHAT-MET groups was stopped, and continued with PBS i.p. injections and plain water for drinking (washout). After this washout, BW gain started to increase in the RIPHAT-MET groups. Interestingly, the BW gain of the RIPHAT-MET+26C11 was already higher compared to the RIPHAT-PBS+MET before and the washout and remained higher also thereafter. At the end of the study, the BW gain of the RIPHAT-MET+26C11 was comparable to the RIPHAT-H2O, while the BW gain of the RIPHAT-MET+PBS increased only slightly. This leads to the conclusion that the administration of NI-203.26C11, on top of metformin administration, has a beneficial effect on the body weight gain compared to metformin alone.

As expected, the fasting glucose (FG) levels of the RIPHAT rat model increased compared to the WT rats over the course of the study. [24 50] All treatments reduced FG in the RIPHAT rats compared to the RIPHAT rats without treatment over the course of the study. There was no difference in FG observed between the antibody NI-203.26C11 and NI-203.11B12. The effect of reduced FG by the antibody NI-203.26C11 was also observed in previous studies. These findings support the idea that the treatment with human derived antibodies targeting IAPP lead to a reduction in the FG by improving beta cell function. [49 50 52]

The FG of the RIPHAT rats, which were treated with metformin, was reduced compared to the control group. This can be explained by the glucose lowering effect of metformin. [41 42]
After 28 weeks of the study, the treatment in the RIPHAT-MET groups was stopped. Six weeks after the washout was performed, fasting glucose was increased in the RIPHAT-MET groups and was comparable to the antibody treatments. It seems that the glucose-reducing effect is only observed during metformin administration but vanishes once the treatment stopped.

As described in the literature, RIPHAT rats show a slightly impaired glucose tolerance starting at an age of 12 weeks. [25] In the present study, the progression of T2DM was slowed down by the antibody treatments, while severe glucose intolerance developed in the RIPHAT rats without treatment over the course of the study. The two antibodies NI-203.26C11 and NI-203.11B12 improved both the glucose tolerance and the AUC of glucose. Between these two treatments, no significant difference was observed. The enhancement of glucose tolerance and AUC of glucose by the antibody NI-203.26C11 was also observed in previous studies. [49 50] In the current study, both antibodies slowed down the progression of glucose intolerance, thereby confirming that targeting IAPP aggregates is of therapeutic value.

The treatment of metformin alone or combined with the antibody NI-203.26C11 lead to a strongly improved glucose tolerance and to a reduced AUC of glucose compared to the RIPHAT rats without treatment. The reduced progression of glucose intolerance was observed from 4 until 26 weeks of the study. The observed decreased glucose levels are an expected effect of metformin during the oGTTs. [30] However, six weeks after the washout, the effect of metformin was not present anymore.

The fasting insulin (FI) concentrations tended to be increased in the treatment groups which received one of the human-derived antibodies, compared to the RIPHAT-H2O+PBS or RIPHAT-MET+PBS groups. Interestingly, the FI of the RIPHAT-H2O+11B12 group was significantly increased compared to the RIPHAT-MET+PBS group at several time-points over the course of the study. Further investigations need to be done to fully understand the reasons for this increase. The FI values were increased by both antibodies, NI-203.26C11 and NI-203.11B12, confirming the results obtained with the NI-203.26C11antibody in previous studies. [49 50]

The insulin response seems to be enhanced by the therapy with the antibody NI.203.11B12 and NI.203.26C11 compared to the control group, as already shown previously. The histological examination in previous studies showed a prevention of the loss of beta-cells by the antibody NI-203.26C11 compared to the control RIPHAT rats. [49 50] A similar effect was to be expected also in the current study and as results of the treatment with the antibody NI-203.11B12. However, histological examination is currently ongoing thus no histological data are available at this time.

The insulin values of the combination treatment, metformin and NI-203.26C11, were increased, while the insulin values of the RIPHAT rats treated with only metformin were comparable to the control RIPHAT rats. Thus, no improvement of the insulin values by metformin alone during the oGTT were observed. That metformin has no protective effect on pancreatic beta cells and consequently no influence on the insulin response had also been described by Matveyenko. [52] Interestingly, improved insulin values were also shown after the administration of the antibody NI-203.26C11 and metformin was stopped in the RIPHAT-MET+26C11 group (washout). It seems that a prolonged treatment effect can be observed by the administration of the antibody NI-203.26C11. The prevention of beta-cell loss, even after the administration of metformin and NI.203-26C11 in the RIPHAT-MET groups was stopped, is an important advantage in this T2DM therapy.

Limitations of the present study are given by the development of T2DM in this rat model. The diabetic phenotype is mainly due to a defective insulin secretion, but not to insulin resistance, which is also an important factor in T2DM. However, the RIPHAT animal model is the only rat diabetes model that mimics the important characteristics of T2DM, which is the formation of amyloid deposits in pancreatic islets. Therefore, despite some disadvantages, it is a very useful tool to test the different therapies. [25 52]
Another limitation of our approach is given due to the metformin administration in the drinking water. The metformin intake is dependent on the water amount of the respective rat, which does not always have to be directly dependent on body weight. However, other ways of application like oral gavage would not be practicable over a long study period.

To summarize, the human-derived antibodies NI-203.26C11 and NI-203.11B12 showed to be well-tolerated and proved their therapeutic efficacy by improving fasting glucose, glucose tolerance, insulin responses and BW gain in treated RIPHAT rats. The results of the current and previous studies support the idea that targeting hIAPP aggregates by antibodies is a very promising treatment strategy.

Combination therapies with metformin, a frequently used glucose-lowering drug which mainly affects insulin sensitivity, are very useful in the treatment of T2DM. [31] In the current study, the human-derived antibody NI-203.26C11 on top of metformin improved the fasting glucose and the glucose tolerance. Before, but especially after the washout was performed, we could observe that the BW gain and insulin values with the combination therapy are improved compared to only metformin treatment in RIPHAT rats. It seems that the insulin response, which reflects the beta-cell function, was still improved by the combination treatment. This combined treatment, which targets two different pathophysiological features of T2DM, could be used as a new treatment strategy in the T2DM therapy.

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8 Annex

8.1 Table list

8.2 Figure list

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